Chapter 5 Final discussion

The majority of recent large-scale sequencing studies of *E. coli* have focused on the most prevalent and highly MDR clone, ST131, and have aimed to find factors responsible for its success as a pathogen [135, 153, 159]. Rather than focusing on a single ST or MDR clone, our study took a longitudinal approach, to capture the diversity of *E. coli* causing bacteraemia in a single hospital over a five-year period to provide basic understanding of E. coli population structure, both from invasive and carriage isolates.

By sequencing randomly more than 650 invasive and carriage isolates over 5 years, our study revealed that there was high genetic diversity in *E. coli*, with member of all classic ECOR phylogroups, comprised of 117 different STs or 15 BAPs clusters. Although exhibiting high diversity, at the same time, all the major STs causing BSIs in Vietnamese patients typically mirror the diversity of ExPEC all over world, including ST131, ST73, ST95, ST69 and ST1193 as dominant STs [101, 105]. These STs have been known for a long time as ExPEC associated with BSIs and UTIs [102, 160]. Interestingly, among these STs, ST131 and ST1193 is common in both invasive and carriage while ST73 and ST95 only found in BSI samples.

Stratifying by year, we have shown that the population structure of dominant *E. coli* STs is stable overtime, with in general the proportion of each STs remaining relatively stable every year. This in itself is an extremely important observation to frame the work done to reduce infection at HTD. The only exception was the introduction of ST1193 in 2011, but once established the population regained stability again. This phenomenon was also observed in the 10-year collection of BSI *E. coli* in United Kingdom [161]. Since majority of BSI were present on admission and so community acquired, these support the hypothesis that there was a high prevalence of carriage of these ExPEC in healthy individuals outside the hospital and in the community. This is particularly significant for immunocompromised patients and patients with other severe comorbidities or unhealthy lifestyles, as we have shown.

As mention above, our study highlighted the introduction of ST1193 in causing BSIs as well as being present in carriage samples in Vietnamese patients. ST1193 was a fluoroquinolone (FQ) resistant clone found as early as 2007 in both human and companion animals (dogs) in Australia [162]. Isolates between those two groups shared the same virulence genes as well as AMR genes, suggesting it was shared between human and animal. ST1193 has also been documented as non-lactose fermenting FQ resistant *E. coli* causing UTIs in Korea [163], being the second most prevalence ST. In the United States, ST1193 was rare, accounted for only 0.9% FQ resistant isolates. However, ST1193 was recently shown to be amongst the top STs associated with community onset infection in 30 county hospitals across China, from 2010 to 2011 [106]. Apart from exhibiting FQ resistant phenotype, ST1193 emerged as a clone that harboured different $bla_{\text{CTX-M}}$ including $bla_{\text{CTX-M-15}}$, $bla_{\text{CMY-2}}$ [164] and $bla_{\text{CTX-M-55}}$ [165].

ST1193 in our collection interestingly, although FQ resistant, harboured a different *bla*$_{CTX-M}$ (*bla*$_{CTX-M-27}$). Collectively, having the same virulence repertoire as ST131, being able to colonize human gut, plus FQ resistant and ESBL-producing might explain the successful clonal expansion of ST1193 in Vietnam. However, further studies combing genomics and epidemiological will be needed to confirm if this is also replicated across and outside of Asia.

Our data confirm that the global emergence of ST131 as the most prevalent ST causing BSIs is also replicated in our hospital and seems to be much more diverse than previously thought. Previous reports [135] have indicated that ST131 isolated from across the globe is FQ resistant and also harbours *bla*$_{CTX-M-15}$. However, in our collection, ST131 did not only harbour *bla*$_{CTX-M-15}$ alone, but also carried plenty other CTX-M variants such as *bla*$_{CTX-M-14}$, *bla*$_{CTX-M-27}$ and *bla*$_{CTX-M-55}$. This highlights the versatility of ST131 in acquiring and maintaining MDR plasmids, in comparison to the other 2 most common ExPECs: ST73 and ST95. The differences observed between the AMR profiles of ST131/ST1193 and ST73/ST95 lead to another interesting point. Since we were unable to find ST73 and ST95 in carriage isolates, we speculate that these two STs might colonise another habitat outside of human gut. The gut and urinary tract are very distinct environments in terms of the availability of nutrients, immunological control, and the population of resident microbes [166]. Since only a small proportion of bacteria that have siderophore systems can live in the urethra [167], perhaps these UPEC clones ST73 and ST69, do not have a chance to encounter other wider community of bacteria including MDR one as in the gastrointestinal tract. Therefore, there are not many chances to pick up mobile genetic elements like plasmids and transposon that carried resistant gene around as ST131.

Although our work in Chapter 4 included a relatively small sample size of 28 patients, this is the first report using WGS to confirm that the majority of BSIs patients (57%) are infected with *E. coli* that are indistinguishable or almost indistinguishable from the dominant strain(s) colonising their guts. This implies that patients were infected with carriage isolates already colonizing their body sites and not through hospital acquired infections as we had initially hypothesised. However, there were exceptions by which haft of the cases were different ST between blood and carriage. For a significant proportion of these isolates we showed these patients had UTI infections, sometimes with *E. coli* that had matching AMR patterns. Although this is perhaps strong circumstantial evidence that their BSIs might originate from prior UTIs we could not confirm this route of infection because the isolates were not stored. If that was true, it will inform future study design by showing that positive urine samples from other bodily sites are critical in order to fully understand colonisation and subsequently invasive infection of *E. coli*. If there were to be a future study, we would collect and sequence all paired *E. coli* isolated from blood, urine and rectal swab on admission from each patient and at a larger scale. By comparing the distribution of STs between different body sites, in the future we might be able to predict which STs can cause BSIs and which STs is just limited to cause bladder infection and also study the interaction of multiple STs coexist in the gut.

Now knowing that colonizing of ExPEC strains such as ST131 and ST1193 pose high risk to subsequently infection in immune compromised patients, either through bacterial translocation in the gut or by seeding bacteria from the gut to the urethra and subsequently bladder infection in the elderly, there are several approaches can be implemented to prevent BSIs infection. The first one is by decolonizing or limiting the presence of these ExPEC and MDR STs in high risk patients. Randomized clinical trial with *Lactobacillus plantarum* has showed that it can prevent 42% of sepsis cases in newborn between control and cases [168]. In term of preventing UTIs, asymptomatic bacteriuria *E. coli* strain 83972 has been used to colonized the urethra and compete with virulent UPEC [169, 170]. Other approach includes treating the gut with sugar molecules resemble sugar structure located on gut epithelial cells that *FimH* can bind, hence also reducing the colonization of *FimH* producing *E. coli* [171]. Since possessing P fimbriae and yersiniabactin has been shown, by us and others, to be the important virulence factors for *E. coli* invasive disease, developing vaccines against these 2 candidates could be a potential approach as well [172, 173].

Our study has several limitations. First of all, because this is a retrospective study we had little control on the sampling strategy either for the random samples or the matched samples from the same patient. We also only had access to rectal swab and blood samples and yet it is clear that other sample such as urines were collected for some patients with UTIs, but unfortunately they were not stored. Also, some of the patient meta data was predicted, for example liver disease was inferred due to patient care and was not through laboratory confirmed testing such as the AST liver function test. The same was true of the CD4 count for HIV status.

Another limitation of the study was the in ability to assemble plasmids from WGS data. In our dataset, we observed substantial diversity of AMR genes and plasmid replicon types, but due to the nature of short reads sequence produced by Illumina sequencing as well as the presence of repetitive elements such as transposases, we were unable to reconstruct the full plasmid structure and the detailed association between certain type of Inc plasmid and certain AMR genes as well as the genetic backbone surround the genes. One plasmid can harbour multiple replicon types apart from their original replicon, and multiple plasmids may co-exist in one cell. This makes it technically challenging to infer total numbers of plasmids based on total replicon types alone. We also used plasmidSPADES [126] to assemble plasmid (data not shown), however, plasmidSPADES operates by comparing read coverage difference between chromosome and plasmid DNA. Since big plasmids (> 90 kb) are not always present in high copy number, they are present as equivalent read depth to the chromosome and therefore are not recognized by plasmidSPADES. Hence these contigs often remain fragmented plasmicidic contigs. In the next study, we would like to employ Long read sequencing also used to elucidate the diversity of AMR genes each clone carried and build as a framework of to track plasmid movement between Enterobacteriace species, especially in our ICU collection.

In conclusion, the success of certain STs is a multifactorial phenomenon including possessing certain virulence genes (attachment, invasion, iron acquisition, toxin producing) and harbouring AMR genes as selective advantage against antimicrobial treatment. Strain that are capable of doing both things, such as ST131 and ST1193, will continue to account for a high proportion of BSIs cases.